ELSEVIER

International Journal of Pharmaceutics 161 (1998) 15–21

international journal of pharmaceutics

Reaction of oxidized glutathione with *cis*- and *trans*-diamminedichloroplatinum(II) and their aquated complexes through the disulfide bond

Naoko Ohta [a,*], Kenji Inagaki [b], Hiroyuki Muta [a], Toshihisa Yotsuyanagi [a], Takakazu Matsuo [a]

[a] *Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467, Japan*
[b] *Laboratory of Chemistry, Seirei Christopher College of Nursing, 3453, Mikatabara-cho, Hamamatsu 433, Japan*

Received 22 April 1997; received in revised form 30 May 1997; accepted 18 September 1997

Abstract

The conjugation of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) by reduced glutathione (GSH) has been reported in several studies. In this study, oxidized glutathione (GSSG) was found to interact with platinum complexes through the S–S bond. The following platinum complexes were used: *cis*-DDP, *trans*-DDP and their aquated complexes. The second-order rate constants for the reactions of *cis*-DDP and its aquated complexes with S–S in GSSG at pH 7.4 and 37°C were 4.12×10^{-3} and 3.56×10^{-2} M^{-1} s^{-1}, respectively. The rate constants for the reactions of *trans*-DDP and its aquated complexes with GSSG were 1.59×10^{-2} and 1.24×10^{-1} M^{-1} s^{-1}, respectively. In order to compare the reactivity of GSH with GSSG, the rate constant for the decrease in GSH sulfhydryl by *cis*-DDP and its aquated complexes were obtained as 7.11×10^{-2} and 2.13×10^{-1} M^{-1} s^{-1}. The rate constant for the reaction of GSH toward *cis*-DDP was 17-fold larger than that of GSSG, while a 6-fold larger rate constant was obtained for the aquated complexes of *cis*-DDP. *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$, the main species at pH 4.5, showed a 2-fold larger rate constant than *cis*-$[Pt(NH_3)_2Cl(OH)]$ at pH 9.5. The difference between the two pHs may reflect that water is a better leaving group than the hydroxide ligand. These results suggest that GSSG may be involved in the conjugation of *cis*-DDP like GSH. © 1998 Elsevier Science B.V.

Keywords: *cis*-Diamminedichloroplatinum(II); Cisplatin; Disulfide bond; Reduced glutathione; Oxidized glutathione; Drug interaction; Metabolism

* Corresponding author. Tel.: +81 52 8363454; fax: +81 52 8352143; e-mail: naokoota@phar.nagoya-cu.ac.jp

PII S0378-5173(97)00323-2

1. Introduction

Cisplatin, *cis*-diamminedichloroplatinum(II) (*cis*-DDP), has been widely used in the anticancer treatment of solid tumors, including testicular, ovarian, head and neck (Chu, 1994). However, treatments with platinum-containing drugs and alkylating agents are often associated with a natural or an acquired resistance for these drugs, which is a major clinical problem. The mechanisms responsible for the development of the resistance involve reduced accumulation of the drug, increased detoxification by glutathione and metallothionein and increased DNA repair (Timmer-Bosscha et al., 1992). Aquired resistance observed clinically is considered to be associated with the combination of more than a single mechanism.

Glutathione, a major component of cellular non-protein thiol, is involved in diverse biological functions such as metabolism, catalysis, transport, and the protection of cells against reactive oxygen species, peroxidase, and xenobiotics (Meister, 1991). Tumor cell lines resistant to *cis*-DDP and alkylating agents are often accompanied by an increase in the cellular glutathione level, thus retarding the amount of platinum that reaches the DNA by binding to glutathione. During the formation of the glutathione conjugate with *cis*-DDP, the reduced form of glutathione (GSH) has been shown to be predominantly involved (Dedon and Borch, 1987). However, little information is available about reactivity between the oxidized form of glutathione (GSSG) and *cis*-DDP. In previous studies, *cis*- and *trans*-DDP induced significant changes in the structure of several plasma proteins which may result from disulfide (S–S) bond cleavage (Ohta et al., 1995a,b; Chen et al., 1994). A similar result was obtained in studies of GSSG and platinum complexes. This is the first study to show the conjugation of *cis*-DDP by GSSG via the S–S bond.

2. Materials and methods

2.1. Materials

cis- And *trans*-DDP were purchased from Aldrich (Milwaukee, WI) and used as received. 2-Nitro-5-thiosulfobenzoate (NTSB) was synthesized. All other chemicals were of analytical grade.

2.2. Reaction of platinum complexes with glutathione

cis- Or *trans*-DDP was dissolved in a 0.5 M $NaClO_4$ solution containing 0.1 M NaCl. The pH of the solutions were adjusted by adding 0.5 N NaOH or HCl solution. NaCl (final concentration of 0.1 M) was added to inhibit the aquation reaction (Miller and House, 1990). The solution was mixed with an excess amount of the platinum complex to glutathione (GSSG or GSH) where the final glutathione concentration was adjusted to 2×10^{-5} M. Incubations were performed at 37°C for appropriate periods, and were protected from light.

The reactions of the aquated forms of *cis*- or *trans*-DDP with glutathione were conducted after the aquation of the respective platinum solution. The platinum complex solutions in 0.6 M $NaClO_4$ without NaCl were allowed to stand for 48 h at 25°C before mixing with glutathione. The rate of *cis*-DDP hydrolysis was measured by following the disappearance of the absorption at 300 nm (not shown). The obtained pseudo first-order rate constant was 2.5×10^{-5} s^{-1}, which was similar to the previously reported value of 2.5×10^{-5} s^{-1} in water and to 2.7×10^{-5} s^{-1} in 0.5 M $NaClO_4$ solution (pH 6.0) at 25°C (Bose et al., 1984). As the hydrolysis rate of *trans*-DDP was shown to be comparable with *cis*-DDP (Bancroft et al., 1990), the standing period for aquation, 48 h, is enough to generate the monohydroxo or monoaqua complexes of *trans*-DDP at 25°C. During incubation with glutathione, the second hydrolysis of these monoaquated forms may proceed to give the diaquated form ($[cis\text{-}Pt(NH_3)_2(H_2O)(OH)]^+$ and a trace amount of $[cis\text{-}Pt(NH_3)_2(OH)_2]$ and $[cis\text{-}Pt(NH_3)_2(H_2O)_2]^{2+}$) (Miller and House, 1990). The reactions of GSH with *trans* isomers were not conducted in this study, since the rate constant was obtained in the literature (Dedon and Borch, 1987). Thus, during the reaction with the aquated platinum complex, several species will be involved. In this study, $HClO_4$/NaOH was used as

the ClO_4^- ion has weak nucleophilic properties while phosphate, tris or other buffer ions could easily chelate with Pt(II).

2.3. Determination of S–S bond and sulfhydryl group

The S–S bond of GSSG was determined as described previously (Ohta et al., 1995a). An 1.5 ml aliquot of 'NTSB assay solution' was added to 2 ml of the incubation mixture containing GSSG. After 5 min, the absorbance at 412 nm was monitored against an appropriate blank. The concentration of the S–S bond was calculated by the amount of 2-nitro-5-thiobenzoic acid formed (Riddles et al., 1983). The sulfhydryl concentration of GSH was also determined by the method of Riddles et al. (1983).

3. Results

3.1. S–S bond decrease in GSSG by cis-DDP and its aquated form

To test the possibility that GSSG could interact with *cis*-DDP or its aquated form through the S–S bond, GSSG was incubated with them. An excess amount of *cis*-DDP or its aquated form was added to GSSG at pH 7.4 and 37°C. In order to inhibit aquation of *cis*-DDP, 0.1 M NaCl was added to the reaction medium. Fig. 1 shows the time courses of the decreased S–S concentration in the reaction mixture in which the molar ratio of the platinum to GSSG varied from 5 to 30. The S–S bond of GSSG was shown to be almost completely diminished after about 450 h by 30-fold *cis*-DDP over GSSG. Meanwhile the S–S decrease due to the aquated *cis*-DDP, as shown in Fig. 2, was faster than *cis*-DDP and almost finished after about 35 h, where the ratio of platinum to GSSG was 40. In the control experiment without the platinum complex, the S–S concentration was not changed over the incubation period.

The decrease in the S–S bond followed pseudo first-order kinetics at least up to two halflives (not shown). In the presence of excess amounts of platinum over GSSG, the pseudo first-order rate constants, k_{obs}, were obtained. As shown in Fig. 3, the plots of k_{obs} versus the *cis*-DDP concentration gave a linear response, demonstrating the

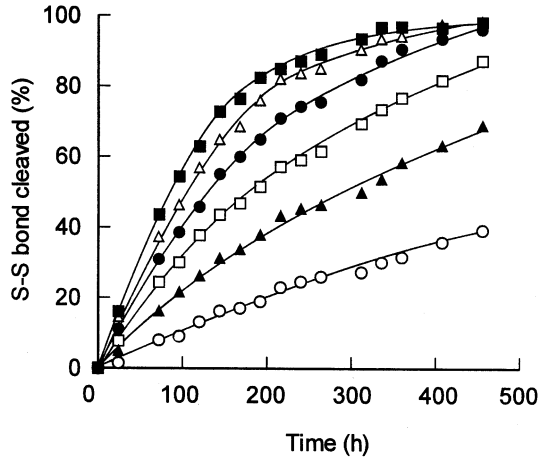

Fig. 1. Time course of disulfide bond change of GSSG by *cis*-DDP. GSSG was incubated with various concentrations of *cis*-DDP at 37°C in 0.5 M $NaClO_4$ containing 0.1 M NaCl (pH 7.4). The GSSG concentration was 20 μM. The *cis*-DDP concentration was (○) 0.1, (▲) 0.2, □ 0.3, (●) 0.4, (△) 0.5, and (■) 0.6 mM.

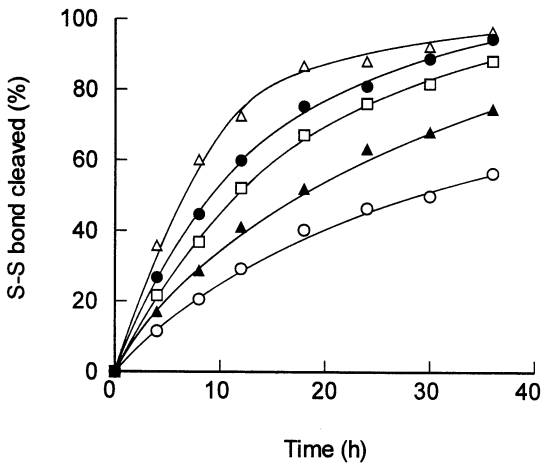

Fig. 2. Time course of disulfide bond change of GSSG by aquated complexes of *cis*-DDP. GSSG was incubated with various concentrations of *cis*-DDP aquated complexes at 37°C in 0.6 M $NaClO_4$ without NaCl (pH 7.4). The GSSG concentration was 20 μM. The concentration of the aquated complex of *cis*-DDP was (○) 0.3, (▲) 0.4, (□) 0.5, (●) 0.6, and (△) 0.8 mM.

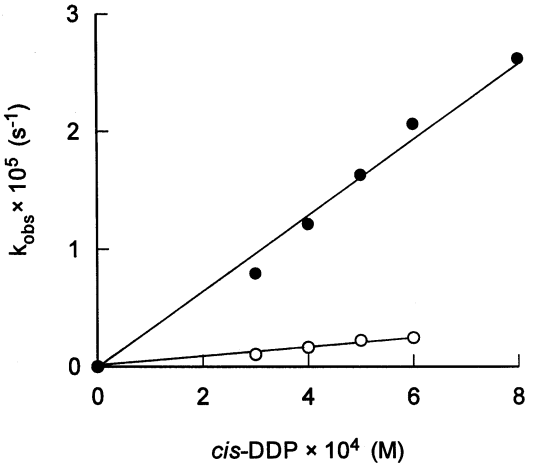

Fig. 3. Relationship between pseudo first-order rate constant and *cis*-DDP or its aquated complex concentration. (○) *cis*-DDP, (●) aquated complexes of *cis*-DDP.

reaction mechanism described in Eq. (1). The second-order rate constant for the decrease in the S–S bond, k_1, can be derived according to Eq. (3).

$$\underset{A}{\text{GSSG}} + \text{Pt } \underset{B}{\text{complex}} \xrightarrow{k_1} \underset{P}{\text{Product}} \quad (1)$$

$$-\frac{d[A]}{dt} = k_{obs}[A] \quad (2)$$

$$k_{obs} = k_1[B] \quad (3)$$

Fig. 3 shows the plots according to Eq. (3) for the reactions of GSSG with *cis*-DDP or its aquated form. The second-order rate constants were obtained from the slope of the lines in Fig. 3; k_1 of the aquated form of *cis*-DDP, 3.56×10^{-2} M^{-1} s^{-1}, was 8.6-fold larger than that for *cis*-DDP, 4.12×10^{-3} M^{-1} s^{-1}, as summarized in Table 1.

3.2. S–S bond decrease in GSSG by trans-DDP and its aquated form

Trans-DDP, the inactive *trans* isomer of *cis*-DDP, also induced a decrease in the S–S bond of GSSG. Time courses of the S–S decrease by *trans*-DDP and its aquated form are obtained where the ratio of platinum and GSSG changed from 5 to 22.5. The change caused by *trans*-DDP was faster than that by *cis*-DDP. 200 h was enough to complete the reaction over the ratio of 20. The second-order rate constants, obtained from the plots according to Eq. (3), are summarized in Table 1 .

3.3. SH decrease in GSH by cis-DDP and its aquated form

The reactivity of GSH toward *cis*-DDP or its aquated form was compared with GSSG. In the presence of excess platinum over GSH, the sulfhydryl content was monitored at pH 7.4 and 37°C. The decrease in SH concentration was induced by complexation with *cis*-DDP or its aquated complexes, respectively.

cis-DDP has been shown to react with GSH; a bis adduct and its polymeric form were isolated by Odenheimer and Wolf (1982), Dedon and Borch (1987), and Ishikawa and Ali-Osman (1993) at a GSH to *cis*-DDP molar ratio of 2. The incubation of GSH with the excess *cis*-DDP aquated complexes in this study would likely produce the equimolar adduct mainly, thus the second-order rate constant for the complexation of GSH and *cis*-DDP was derived according to Eqs. (1)–(3) where GSSG was displaced by GSH. These rate constants are listed in Table 1. The rate constant for *cis*-DDP and its aquated complexes were obtained as 7.11×10^{-2} and 2.13×10^{-1} M^{-1} s^{-1}, respectively. The rate constant for the reaction of GSH toward *cis*-DDP was 17-fold larger than that of GSSG, while a 6-fold larger rate constant was obtained for the aquated complexes of *cis*-DDP.

Table 1
Second-order rate constants for the reactions between platinum complexes and GSSG or GSH at pH 7.4 and 37°C

Platinum complex	GSSG rate constant (M^{-1} s^{-1})	GSH rate constant (M^{-1} s^{-1})
cis-DDP	4.12×10^{-3}	7.11×10^{-2}
Aquated complexes of *cis*-DDP	$3.56 \times 1O^{-2}$	2.13×10^{-1}
trans-DDP	1.59×10^{-2}	3.91*
Aquated complexes of *trans*-DDP	1.24×10^{-1}	N.D.

N.D., not determined.
*Dedon and Borch (1987).

Table 2
pH-dependence of the second-order rate constants for the reaction between monoaqua complexes of *cis*-DDP and GSSG

pH	Rate constant (M^{-1} s^{-1})
4.5	5.14×10^{-2}
5.5	5.14×10^{-2}
6.5	3.95×10^{-2}
7.4	3.56×10^{-2}
8.5	2.64×10^{-2}
9.5	2.63×10^{-2}

0.5 M $NaClO_4$ solution without NaCl at 37°C.

3.4. Effect of pH

In order to investigate the reactivity of GSSG under conditions similar to that within a cell, GSSG was incubated with the *cis*-DDP aquated complexes at various pHs including the weak acidic pH intracellularly encountered. The pH did not change during the course of the reaction. The second-order rate constants were obtained in the range of pH 4.5 to 9.5, as shown in Table 2.

3.5. Effect of NaCl concentration on the S–S bond decrease by cis-DDP

To assess the hypothesis that the hydrolyzed species [*cis*-$Pt(NH_3)_2Cl(H_2O)]^+$ or [*cis*-$Pt(NH_3)_2Cl(OH)$] are the reactive intermediates responsible for the binding of platinum to GSSG, chloride was added to the incubations of *cis*-DDP and GSSG. If this hypothesis is valid, a high concentration of chloride would completely prevent the reaction. However, the S–S decrease in GSSG was observed even in 3.4 M NaCl solution to an extent similar to the low Cl^- concentration (Fig. 4).

4. Discussion

In most cells, the concentration of glutathione is in the range 0.5–10 mM, and outside the cell it's of μM order (Lempers et al., 1988; Ishikawa and Ali-Osman, 1993). Glutathione is one of the nucleophiles that *cis*-DDP would encounter in the cell. Thus, the detoxification of *cis*-DDP by high levels of glutathione is accepted as one of the mechanisms of the acquired resistance (Timmer-Bosscha et al., 1992). In experimentally developed human ovarian cancer cell lines resistant to *cis*-DDP, the glutathione level increased 13–50 times higher than that in cells without resistance dependent on the *cis*-DDP dose ($30\sim200$ μM) (Godwin et al., 1992). Extensive studies for the conjugation of *cis*-DDP by the reduced glutathione were conducted (Odenheimer and Wolf, 1982; Corden, 1987; Dedon and Borch, 1987; Ishikawa and Ali-Osman, 1993). The disappearance of the S–S bond in GSSG, induced by complexation with platinum complexes observed in this experiment raises the interesting possibility that the inactivation of *cis*-DDP or its aquated species by GSSG as well as by GSH is partly responsible for the glutathione conjugation.

Inside the cell, chloride ion concentration is low (about 4 mM), and the chloride is slowly released from *cis*-DDP by a shift in the equilibrium to the hydrolysis of the first chloride ion. The formation of the hydrolyzed species [*cis*-$Pt(NH_3)_2Cl(H_2O)]^+$ or [*cis*-$Pt(NH_3)_2Cl(OH)$] is a requirement for the binding of platinum to DNA (Corden, 1987). The data summarized in Table 1 shows that the second-order rate constant for the reaction between GSSG with aquated species is 8.6- or 7.8-fold

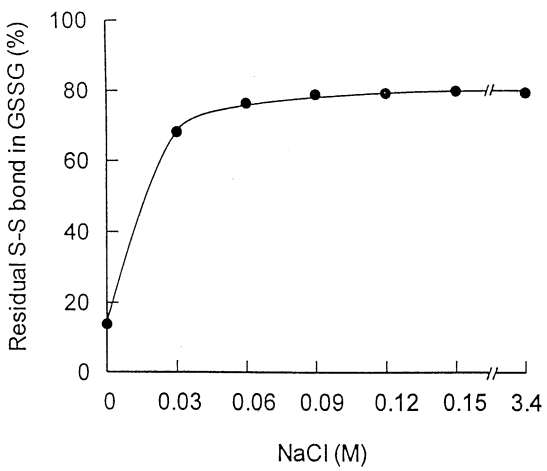

Fig. 4. Effect of NaCl concentration on disulfide bond of GSSG (20 μM) incubated with 0.8 mM *cis*-DDP at pH 7.4 and 37°C. The S–S concentration was determined after 24 h incubation.

larger than that with each parent drug, *cis*- or *trans*-DDP, respectively. The higher reactivity of the aquated form than DDP was observed in the reaction with DNA as well as the S–S bond of GSSG. Although the aquated platinum complexes are known to react with nucleophiles at least 10-fold faster than the parent complex, the observed acceleration of the aquated complex was slightly less than expected (Howe-Grant and Lippard, 1980),

The aquated complexes produced by the first hydrolysis are [*cis*-$Pt(NH_3)_2Cl(H_2O)]^+$ and [*cis*-$Pt(NH_3)_2Cl(OH)$] with a pK_a of 6.85. The second-order rate constant for the reaction between the aquated complexes and GSSG at pH 9.5 was about 2-fold smaller than that at pH 4.5. This result is consistent with the fact that the affinity of OH^- to platinum is higher than that of H_2O, and suggests that water is about a 2-fold better leaving group than the hydroxide ligand (Howe-Grant and Lippard, 1980).

The influence of concentration of NaCl on the decreasing rate of S–S by *cis*-DDP is presented in Fig. 4. Up to 60 mM NaCl, S–S cleavage was sharply decreased, suggesting that the aquated form is more reactive than *cis*-DDP. In the presence of 60, 150 mM and 3.4 M the rates of reaction are indistinguishable; 20% of S–S bonds were cleaved in 24 h at 37°C, pH 7.4. However, the degree of suppression of the aquation reaction is different in these NaCl concentrations. According to Miller and House (1990), at 37°C in pH 7.4 solution containing 60, 150 mM, or 3.4 M NaCl, *cis*-DDP changes to hydrolyzed products of 26.2, 12.2 or 0.6% at equilibrium. The aquation did not correlate S–S cleavage and incomplete suppression of reactivity even in 3.4 M NaCl solution was observed. It has now been generally accepted that *cis*-DDP can react with DNA only after a chloro ligand is replaced by water. Concerning the rate-determining step of *cis*-DDP with sulfur-containing biomolecules, the available data are quite controversial. It has been reported that the chloro hydrolysis is the rate-determining step in reactions of *cis*-DDP with leucine aminopeptidase, with γ-glutamyl transpeptidase, and also with albumin (Dedon and Borch, 1987; Lempers and Reedijk, 1991).

However, it has also been suggested that there may be a direct binding to proteins without prior aquation, and this has been observed with cysteine, GSH, adenosine triphosphatase, and with MT (Corden, 1987; Lempers and Reedijk, 1991). In this case of GSSG, the pathway of the direct binding to GSSG without prior aquatation is not necessarily predominant.

The second-order rate constants for the reaction of *cis*- and *trans*-DDP and their aquated complexes against GSSG or GSH are listed in Table 1. The rate constant for *trans*-DDP and GSH, 3.91 $M^{-1}\ s^{-1}$ is quoted from the literature (Dedon and Borch, 1987). The rate constants for the reaction of *cis*- and *trans*-DDP against GSSG were, 4.12×10^{-3} and $1.59 \times 10^{-2}\ M^{-1}\ s^{-1}$, respectively, and the *trans* isomer showed up 3.8-fold larger than *cis*-DDP. A more remarkable trans effect was shown in GSH, *trans*-DDP (3.91 $M^{-1}\ s^{-1}$) reacted with GSH sulfhydryl 55-fold faster than *cis*-DDP ($7.11 \times 10^{-2}\ M^{-1}\ s^{-1}$) (Table 1). Thus glutathione rapidly binding to *trans* platinum complexes rather than *cis* isomers may be due to the trans-labilizing power of the coordinated ligand. Once bound to a labile site, glutathione will tend to labilize the *trans* chloride ion, facilitating platinum coordination to another site. In the previous studies *trans*-DDP was observed to be more readily incorporated than *cis*-DDP into proteins, such as α_2-macroglobulin, α_1-proteinase inhibitor, human and bovine serum albumin (Gonias et al., 1988) although the authors suggested Cys sulfhydryl or Met residue as the platinum binding site.

In the case of the reaction between GSH and *cis*-DDP, two inconsistent products have been demonstrated by data using IR and NMR, namely the 2:1 and 2:2 complexes (Odenheimer and Wolf, 1982; Appleton et al., 1989). On the other hand, in the case of GSSG, assuming that a 1:1 stoichiometry product is formed, plots between k_{obs} and Pt concentration gave a linear relationship (Fig. 3). This is consistent with the result obtained by a spectrophotometric study suggesting 1:1 product formation (unpublished data).

In inorganic chemistry, the coordination of several metals to GSSG has been studied. Only one

example of the S–S bond cleavage by [PtCl(-dien)]$^+$ was studied by Lempers et al. (1988), of whom Inagaki is one of the authors of this paper. The result and our study suggest the interaction of GSSG via the S–S bond would involve *cis*-DDP, its hydrolyzed complexes and furthermore, the monofunctional adduct of *cis*-DDP bound to DNA within the cancer cell, since [PtCl(dien)]$^+$ is a model compound of the monofunctional adduct formed by DNA and *cis*-DDP, although these platinum complexes are more readily conjugated by GSH rather than GSSG.

Glutathione is usually in the reduced form in the body and less than 5% of the glutathione is in the oxidized form (Ishikawa and Ali-Osman, 1993). Furthermore, the rate constant of the reaction between *cis*-DDP or aquated complexes with GSSG was 17- or 6-fold slower than that with GSH. Therefore, GSH would be mainly responsible for the conjugation and/or metabolism of *cis*-DDP, and the involvement of GSSG with clinical relevance might be ignored.

References

Appleton, T.G., Connor, J.W., Hall, J.R., Prenzler, P.D., 1989. NMR study of the reactions of the *cis*-diammine-diaquaplatinum(II) cation with glutathione and amino acids containing a thiol group. Inorg. Chem. 28, 2030–2037.

Bancroft, D.P., Lepre, C.A., Lippard, S.J., 1990. ^{195}Pt NMR kinetic and mechanistic studies of *cis*- and *trans*-diamminedichloroplatinum(II) binding to DNA. J. Am. Chem. Soc. 112, 6860–6871.

Bose, R.N., Viola, R.E., Cornelius, R.D., 1984. Phosphorus-31 NMR and kinetic studies of the formation of ortho-, pyro-, and triphosphato complexes of *cis*-dichlorodiammineplatinum(II). J. Am. Chem. Soc. 106, 3336–3343.

Chen, D., Ohta, N., Ikeda, K., 1994. Polymerized precipitation of human γ-globulin induced by *cis*-diamminedichloroplatinum(II). Int. J. Pharmaceut. 106, 249–253.

Chu, G., 1994. Cellular responses to cisplatin. J. Biol. Chem. 269, 787–790.

Corden, B.J., 1987. Reaction of platinum(II) antitumor agents with sulfhydral compounds and the implications for nephrotoxicity. Inorg. Chim. Acta 137, 125–130.

Dedon, P.C., Borch, R.F., 1987. Characterization of the reactions of platinum antitumor agents with biologic and non-biologic sulfur-containing nucleophiles. Biochem. Pharmacol. 36, 1955–1964.

Godwin, A.K., Meister, A., O'Dwyer, P.J., Huang, C.S., Hamilton, T.C., Anderson, M.E., 1992. High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. Proc. Natl. Acad. Sci. USA 89, 3070–3074.

Gonias, S.L., Swaim, M.W., Massey, M.F., Pizzo, S.V., 1988. *cis*-Dichlorodiammineplatinum(II) as a selective modifier of the oxidation-sensitive reactive-center methionine in α_1-antitrypsin. J. Biol. Chem. 263, 393–397.

Howe-Grant, M.E., Lippard, S.J., 1980. In: Sigel, H. (Ed.), Metal Ions in Biological Systems, vol. 11. Marcel Dekker, New York, pp. 63–125.

Ishikawa, T., Ali-Osman, F., 1993. Glutathione-associated *cis*-diamminedichloroplatinum(II) metabolism and ATP-dependent efflux from leukemia cells. J. Biol. Chem. 268, 20116–20125.

Lempers, E.L.M., Inagaki, K., Reedijk, J., 1988. Reactions of [PtCl(dien)]Cl with glutathione, oxidized glutathione and *S*-methyl glutathione. Formation of an S-bridged dinuclear unit. Inorg. Chim. Acta 152, 201–207.

Lempers, E.L.M., Reedijk, J., 1991. Interactions of platinum amine compounds with sulfur containing biomolecules and DNA fragments. Adv. Inorg. Chem. 37, 175–217.

Meister, A., 1991. Glutathione deficiency produced by inhibition of its synthesis and its reversal: applications in research and therapy. Pharmacol. Ther. 51, 155–194.

Miller, S.E., House, D.A., 1990. The hydrolysis products of *cis*-dichlorodiammineplatinum(II) 3. Hydrolysis kinetics at physiological pH. Inorg. Chim. Acta 173, 53–60.

Odenheimer, B., Wolf, W., 1982. Reactions of cisplatin with sulfur-containing amino acids and peptides 1. Cystein and glutathione. Inorg. Chim. Acta 66, L41–L43.

Ohta, N., Chen, D., Ito, S., Futo, T., Yotsuyanagi, T., Ikeda, K., 1995a. Effect of *trans*-diamminedichloroplatinum(II) on human serum albumin: conformational changes through partial disulfide bond cleavage. Int. J. Pharmaceut. 118, 85–93.

Ohta, N., Chen, D., Ukai, M., Matsuo, T., Masuda, M., 1995b. Aggregation of γ-globulin by *cis*-diamminedichloroplatinum(II): alteration of Fc region and restoration by diethyldithiocarbamate. Int. J. Pharmaceut. 124, 165–172.

Riddles, P.W., Blakeley, R.L., Zerner, B., 1983. Reassessment of Ellman's reagent. Methods Enymol. 91, 49–60.

Timmer-Bosscha, H., Mulder, N.H., de Vries, E.G.E., 1992. Modulation of *cis*-diamminedichloroplatinum(II) resistance: a review. Br. J. Cancer 66, 227–238.

.